Arsenic Release from Spent Water Treatment Adsorbents: The Role of Iron Reducing Microorganisms

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By

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ABSTRACT

Recent studies have shown that arsenic can be released from spent iron-based sorbent media (IBS) disposed in municipal landfills. The reasons for this release are not completely understood, as there are many chemical and biological processes that may possibly result in this release. The purpose of this research is to understand the biological causes as a result of the dissimilatory reduction of iron oxides by using the iron-reducing bacteria, *Geobacter Metallireducens* GS-15.

GS-15 was first cultured and transferred before being tested with dissolved iron, dissolved arsenic, and solid iron goethite media types. GS-15's electron donor metabolism capabilities were tested. Arsenic release was determined under three amended media conditions with the use of arsenic loaded solid goethite, a media similar to spent IBS. It was determined that with the use of dissolved iron only acetate was utilized as an electron donor; however, this was not observed for the solid iron goethite media, where the utilization of citrate was observed. The dissolved arsenic conditions did not sustain growth of GS-15. Arsenic release in the presence of GS-15 was lower than that observed in abiotic samples when citrate was the electron donor. With acetate as the electron donor and with acetate and the presence of elevated phosphate; however, the release was greater with the presence of GS-15 than that observed in the abiotic samples. Further studies need to be conducted to determine why the reduction of citrate

occurred as this has not been previously observed. Addition studies need to be conducted to understand the release of arsenic with other elevated anion levels to understand fully the impact of iron reducing microbes on the spent IBS media in landfill conditions.

ACKNOWLEDGEMENTS

There are many people who I would like to acknowledge for the instrumental help they provided to me during the course of my research. First, I would like to express my sincerest thanks to my advisor, Dr. John Lenhart. Through the variety of dilemmas I experienced during my research, Dr. Lenhart kindly accepted my constant stopping by his office without an appointment to answer any questions or concerns I had. Dr. Lenhart also provided me with constant advice, assistance, and encouragement for which I am extremely grateful.

I am indebted to Mengling Stuckman for teaching me all of the lab techniques that I know today and being a constant help with anything that I did not understand within this research. I must also thank Lauren Corrigan along with Mengling Stuckman for staying in the lab to sample and run instruments into all hours of the night. I must also thank Lauren Corrigan for always giving me encouragement and a good laugh when I needed it. Finally, I must thank Dr. Paula Mouser for allowing me to us her lab space and never failing to assist and teach me new lab techniques. Thank you all.

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CHAPTER 1: INTRODUCTION

Arsenic (As) is a natural metalloid element abundant within the earth's crust in small concentrations. Arsenic can enter into water supplies and the atmosphere through natural causes such as wind-blown dust, water run-off, volcanic eruptions, weathering, and microorganism release (US Department of Health and Human Services, 2007). In some instances human manufacturing of preserved wood, paints, dyes, along with previously used agricultural fertilizers and feeds, and metal industrial processes have resulted in release of arsenic into the atmosphere and drinking water through ground contamination and water runoff (US EPA, 2011). Some areas are associated with higher arsenic levels as a result of natural causes and/or industrial or past agricultural practices; leaving some populations at a greater risk for arsenic exposure. In Figure 1, one can see that areas in the Central and Western United States are more susceptible to elevated arsenic concentrations in ground water. This is largely due to minerals dissolving from weathered rocks and soils (US Geological Survey, 2011).

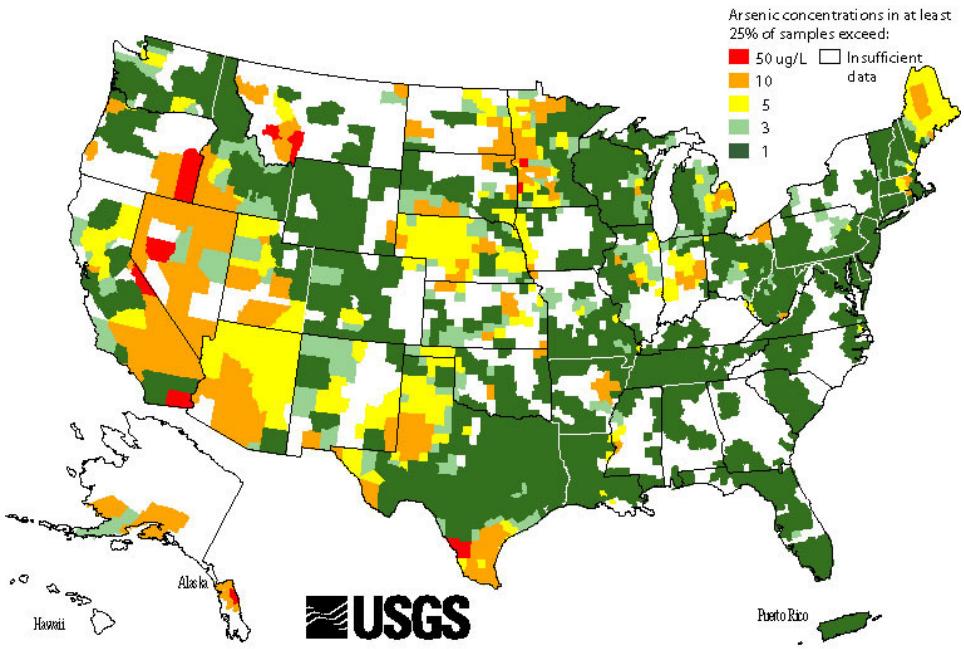

Figure 1: USGS map of arsenic in ground water based on 18,850 ground-water samples (United States Geological Survey, 2011).

Elevated arsenic groundwater concentration is a major health and safety issue as it is classified as a human carcinogen by the Department of Health and Human Services, International Agency for Research on Cancer, and the Environmental Protection Agency (EPA). Detection by humans' senses is limited as it is colorless, odorless, and tasteless. Arsenic exposure is possible through ingestion and inhalation and is characterized by irritation of the intestines and stomach and irritation of the lungs and the throat (US Department of Health and Human Services, 2007). Long-term effects include skin changes such as the development of small corns or warts, changes in blood vessels that are represented through easy bruising, circulatory, peripheral nervous disorders, and an increased risk for liver, bladder, and lung cancers (US Department of Health and Human Services, 2007). Arsenic exposure may also interfere with normal fetal development (US Department of Health and Human Services, 2007).

With these health and safety issues the US EPA adopted the standard of 50 parts per billion (ppb) in 1975 as part of the National Interim Primary Drinking Water Standards (National Research Council, 1999). A 1999 report conducted by the National Academy of Sciences to assess the arsenic levels showed that the arsenic standards would need to be lowered to ensure exposure risks are minimized (National Research Council, 1999). The US EPA amended the Safe Drinking Water Act in June of 2001 by lowering the arsenic maximum contaminant level (MCL) to 10 ppb (US EPA, 2011).

Previous research identified several treatment methods capable of removing As from public supplies (Table 1). Iron-based sorbent media (IBS) has proven to be an effective, economical treatment method as it has the possibility for the highest removal efficiency that can be used in a Point of Use System (US EPA, 2003). Pre-oxidation is a common requirement for the tabulated treatment methods as it is a process that will oxidize all the arsenic present to As (V). Arsenic is commonly present in two ionic forms As (III) and As (V). As (III) is uncharged at neutral pH, causing it not to be treated as readily. In comparison to the two other types of arsenic removal, ion exchange and activated alumina, IBS has the least amount of water quality requirements (Table A1).

Table 1: A list of arsenic treatment technologies that have been suggested by the US EPA (US EPA, 2007).

Arsenic Treatment Technologies	
Activated alumina (AA)	Greensand Filtration
Anion Exchange	Oxidation/Coagulation/Filtration
Iron-Based Sorbent Media	Lime Softening
Mixed Bed Ion Exchange	Reverse Osmosis

IBS can be applied in a Point of Use Treatment allowing for simple operation, low maintenance, low relative cost, small under-the-counter footprint, and high treatment capacity (US EPA, 2003). IBS can also be applied to a series of fixed bed pressure columns that allow for low relative costs, high treatment capacity, and relatively low maintenance (US EPA, 2003). IBS is considered a one-time use media that does not exceed 5 mg/L (~67 mM) of arsenic, which is the toxicity characteristics set forth by regulation 40 CFR 261 – Identification and Listing of Hazardous Waste (MacPhee, 2001). This requirement allows IBS to be disposed of in municipal solid waste landfills as opposed to being handled as hazardous waste.

Disposal of IBS in landfills exposes the media to a variety of competitive anions and varying biological processes that are not tested when determining toxicity characteristics. Studies simulating release under landfill conditions demonstrate release of As in excess of the level set by 40 CFR 261(Yi, 2010) bring to light that further studies need to be conducted to understand which processes are the source of arsenic leaching. At this time, insufficient studies have been performed to understand which of the competitive anions and/or microbial processes are the source of the arsenic leaching. Multiple competitive anions have been reported to inhibit the sorption of arsenic by competing for the adsorption sites present on IBS. Those that have been evaluated include bicarbonate (Holm, 2002), phosphate (Dixit et al., 2003), sulfate (Xu et al., 1988), silicate (Holm, 2002), and natural organic matter (Xu et al., 1991).

It is difficult to ascertain the dominant microbial pathway for arsenic release as three microbial processes have been identified to possibly result in arsenic release: dissimilatory arsenic reduction, iron reduction resulting in possible arsenic release, and a

combination of both dissimilatory arsenic and iron reduction (Oremland, 2003). Studies have shown that reduced forms of arsenic have a lower affinity to IBS media possibly resulting in the release of As (III), which cannot be sequestered easily (Ng, 2004). In addition, studies have observed that the reduction of iron has led to the displacement of arsenic from the solid phase arsenic bearing iron hydroxides although it is still controversial as to whether or not this reduction also allows for arsenic sequestration onto the new iron forms (Yi, 2010). Currently there is research being completed to determine the role that arsenic reducing and arsenic/iron reducing bacteria have on the spent IBS. Therefore, this research will focus on the leaching of arsenic within landfill conditions specifically due to iron reducing microorganisms.

Geobacter metallireducens (GS-15) was chosen for this research, as it is an iron reducing bacterium that can obtain energy for growth by coupling the complete oxidization of organic compounds with the reduction of iron (Lovely, 1993). *Geobacter metallireducens* is a gram-negative motile microbe first isolated in sediments of the Potomac River, Maryland in 1987 ("Basic Science with an Applied Product"). In addition, *Geobacter metallireducens* has been shown to not reduce arsenic (Tadanier, 2005).

In order to determine how *Geobacter metallireducens* affects the mobility of arsenic in landfill leachate a series of tests were performed. Sampling of inoculated growth media amended with dissolved iron (III) citrate were performed to determine the growth period of *Geobacter metallireducens* within synthetic leachate. Sampling of inoculated synthetic leachate with solid goethite were performed to determine the growth period of *Geobacter metallireducens* as limited literature been produced on this.

Sampling of inoculated synthetic leachate with arsenic loaded solid goethite were performed to determine the effects of the iron-reduction on the mobility of arsenic.

CHAPTER 2: EXPERIMENTAL METHODS

2.1 Overview

The research was conducted in a series of three tasks in order to determine the biological effects that *Geobacter metallireducens* (GS-15) has on the release of arsenic from the reduction of iron within arsenic loaded solid goethite (As-E33). First, the GS-15 was cultured and transferred, followed by sampling of inoculated media including dissolved iron (III) citrate (FeCit) and solid iron – goethite (E33) along with dissolved sodium-arsenate. Then sampling of inoculated media of As-E33 was completed. The Environmental Engineering Department Laboratory provided all equipment and materials.

2.2 Culture and Harvesting Procedures

The first task involved culturing GS-15 and transferring the cells to the media type used for analysis. *Geobacter metallireducens* ATCC® 53774™ was purchased from the American Type Culture Collection (ATCC) in Manassas, Virginia. GS-15 was grown in two media types, synthetic leachate and original growth media following ATCC® Media Broth #1768 recipe (Table A2 and A3). Sodium acetate (10 mM) was added as the elector donor and FeCit (10 mM) was added as the elector acceptor.

All procedures were done following anaerobic techniques (Miller, and Wolin. 985-987) using a COY Laboratory Products, Inc. anaerobic chamber and anaerobic gassing station (Figure A1), with N_2: CO_2 at 80:20. Supleco Park's Supelpure O 2.2449 oxygen stripper was used to remove residual O_2 from the system. Initial inoculation was done with 2.5 mL of each media into separate 26 mL serum tubes using rubber stoppers and aluminum crimps. Incubation was done at 30° C in the dark without shaking. Gas phases for the media were 20% CO_2 balanced with N_2. Final pH of the media was approximately 7.0.

Transfers and harvesting of cells were done once the iron reduction was around eighty percent completed; this was determined based on color change of the media due to the reduction of iron from a clear-burnt orange to an opaque white liquid (about 24 hr), (Figure A2 to A4). Cells were preserved in 30% glycerol using a 1:1 ratio and stored in -80 °C. Simple cell transfers were completed into synthetic leachate using a 1:10 dilution (culture: media). This method is used for cell growth rather than inoculation of media for experimental sampling.

For inoculation of experimental samples a bicarbonate wash was completed. Cells were combined from each bottle to produce a single sample before a pellet was produced. Cell pellets were produced by centrifuging at 8000 x g for 20 min and then were washed with 3 mL of bicarbonate (300 mM) and centrifuged again. This process was repeated twice more. The remaining pellet was combined with the required quantity of bicarbonate for the media and absorbance was measured on a Thermo Scientific Genesys 20 spectrometer.

2.3 Batch Test Sampling Procedures

The second task involved sampling media formulated from synthetic leachate with a matrix of electron donor and acceptor conditions (Table 2). The abbreviations listed in Table 2 will be used throughout. The final pH, gas phases, and storage conditions were as described previously. The samples were stored at 30° C in the dark. The E33 samples were the only samples shaken. Anaerobic sampling was done using the anaerobic gassing station discussed previously.

Table 2: Summary of abbreviations for the sampled bottles for each electron donors and electron donors.

	Electron Donor*			Elevated Anions
Electron Acceptor	Acetate (10 mM)	Lactate (10 mM)	Citrate (10 mM)	Phosphate (3.2 mM)
Dissolved iron(III) citrate (10mM)	FeCit – Ace**	FeCit – Lac	FeCit – Cit	
Solid Goethite (E33) (50 g/L)	E33 – Ace	E33 - Lac	E33 – Cit	
Dissolved Sodium arsenate (10mM)	As - Ace			
Arsenic Loaded Goethite (50 g/L)	As-E33 – Ace		As-E33 – Cit	As-E33 – Ace + P
* For each amended synthetic leachate solution, an abiotic bottle was sampled. ** Two biotic bottles were sampled for this solution type				

The FeCit sampling procedure was conducted using seven 125 mL serum bottles with 50 mL of media. One mL samples were taken from each bottle over forty-six hours (iron reduction around eighty percent). Iron concentration was determined using the Ferrozine method (Ferrozine in HEPES) following the methods in Stookey (1970) immediately after sampling. The electron donor metabolism was determined using a

sample in 1:50 dilution (samples: DI water) stored at 4 °C for less than two weeks before measurement on a Dionex ICS-2100 ion chromatography.

For the E33 sampling procedure, six 125 mL serum bottles with 100 mL media and 5 g E33 was used. 4.5 mL samples were taken from each bottle over 10 days. Iron concentration and electron donor metabolism were determined as discussed previously.

For the dissolved sodium arsenate sampling procedure, three 125 mL serum bottles with 100 mL media were used. 8.5 mL samples were taken from each bottle over 10 days. Electron donor metabolism was determined as discussed previously. Arsenic (III) was determined using arsenic speciation cartridges (Meng, 1998) and the arsenic species concentration was determined using Varian Vista AX ICP-AES; samples were stored in 5% nitric acid for at the most one week.

2.4 Arsenic Loaded Iron Solids Procedure

The third task involved sampling 1 L bottles of synthetic leachate amended with As-E33 (50 g/L) ([As] = 5 mM). Refer to Table 2 for the amended electron donors and elevated anions. The As-E33 was synthesized by equilibrating the E33 for three days with shaking at 250 rpm in a media with arsenic (3.13 g/L) and in the background solution described in Table A4. The solution was then centrifuged at 3500 rpm for thirty minutes, followed by washing with 100 mL of distilled water and centrifuged at 3500 rpm for thirty additional minutes. The solids were then air-dried for three days before storage.

The final pH, gas phases, and storage conditions were as described previously. The samples were shaken at 15 rpm. Anaerobic sampling and measurements follow the previously described method for the dissolved sodium arsenate samples.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Culturing and Transfer of the Cells

Culturing and growth of GS-15 was found to be successful using the synthetic leachate compared to the original growth media, as iron reduction was more rapid with the former. A few issues were encountered during the culturing of the sample from ATCC®, the most significant being that the original culture from ATCC® did not grow in the original growth media after thirty days. When it is stated that growth should occur after three to five days.

Therefore, upon receiving a second culture, half of the sample was placed in the original growth media and half in the synthetic leachate media. The synthetic leachate media sustained growth and the bacteria were able to completely reduce the iron in approximately one day, while the original growth media did not completely reduce the iron for twenty-five days. It can then be concluded that the first culture was not viable, as growth did end up occurring for the second culture in the original growth media. Pictures of synthetic leachate and original growth medias at zero hour and after one day are shown (Figure 2). As one can see, significant reduction, as credited through color change, occurred in the synthetic leach media compared to the original growth media.

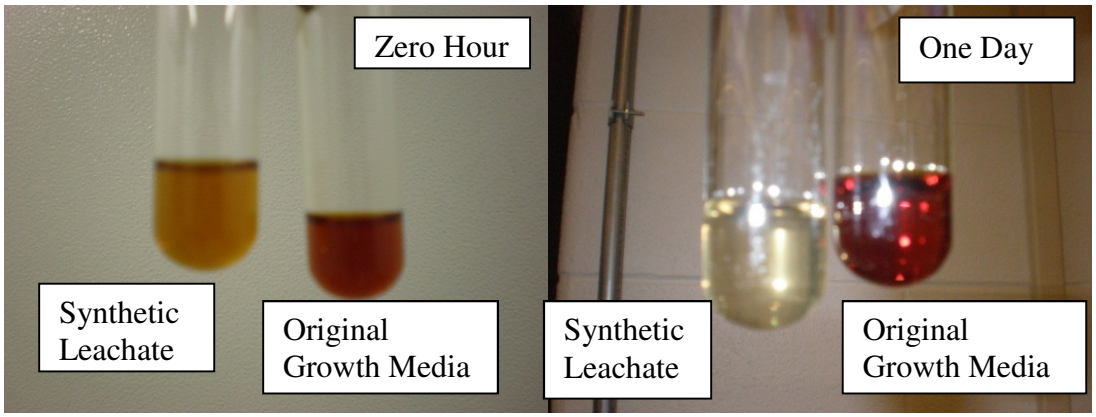

Figure 2: Pictures of the two medias compared side by side at zero hour and at day one.

Cell transfers done using the bicarbonate wash were shown to have a successful amount of cells transferred as growth was sustained in the inoculated medias. However, it was found that during the bicarbonate washing that the cell density was decreased, as inoculated media would take around forty-six hours to completely reduce the iron compared to simple cell transfers done previously. This was believed to be the result of the trauma encountered during centrifuging, the anaerobic conditions not being sustained completely within the centrifuge tubes, and loss of cells through wash fluid. A flow chart of cell transfers, harvest, and inoculation done throughout the experimental period can be seen in Figure A5.

3.2 Sampling of Dissolved Iron (III) citrate with Three Electron Donors

This sampling process was used to determine electron donor metabolism and growth rates for GS-15 under conditions studied for comparison to data present in literature. These samples were inoculated with Son-5A, (Figure A5). The only electron donor found to be metabolized by GS-15 was acetate (Figure A6) which is supported by the literature (Lovley 1993). The stoichiometric equation for this metabolism was

assumed to be that represented in Equation 1 (Lovley and Phillips, 1988). This assumption was based on experimentally observed white precipitate that appeared after complete iron reduction in the media was achieved. These results mimic those seen in Lovley and Phillips (1988), which determined this precipitate to be vivianite. Following this assumed stoichiometry for the acetate metabolism, the iron reduction only achieves approximately fifty percent of its ideal value; however, some of the acetate would be metabolized for cell synthesis (Lovley and Phillips 1988).

Equation 1:

$$CH_3COO^- + 8\,Fe^{+3} + 3\,H_2O \rightarrow 8Fe^{2+} + HCO_3^- + CO_2 + 8\,H^+$$

Iron reduction, seen in Figure 3, was determined by a ratio of iron (II) over total iron. Iron concentrations (Figure A7) for acetate metabolism show a steady increase in iron (II) production after the initial lag phase of ten hours. The observed decrease in the iron reduction that occurs at thirty-seven hours coincides with an increase of acetate; similar trends were seen in preliminary growth curves (Figure A8). Comparison of this reduction rate with those seen in Lovley and Phillips (1988) shows that their results present a faster rate, as iron reduction was completed in approximately twenty-four hours and showing a lag phase of only five hours and there was no decreases of iron (II) production. This may be due to the differences in cell washing methods and the concentration ratios of electron donor to iron (III).

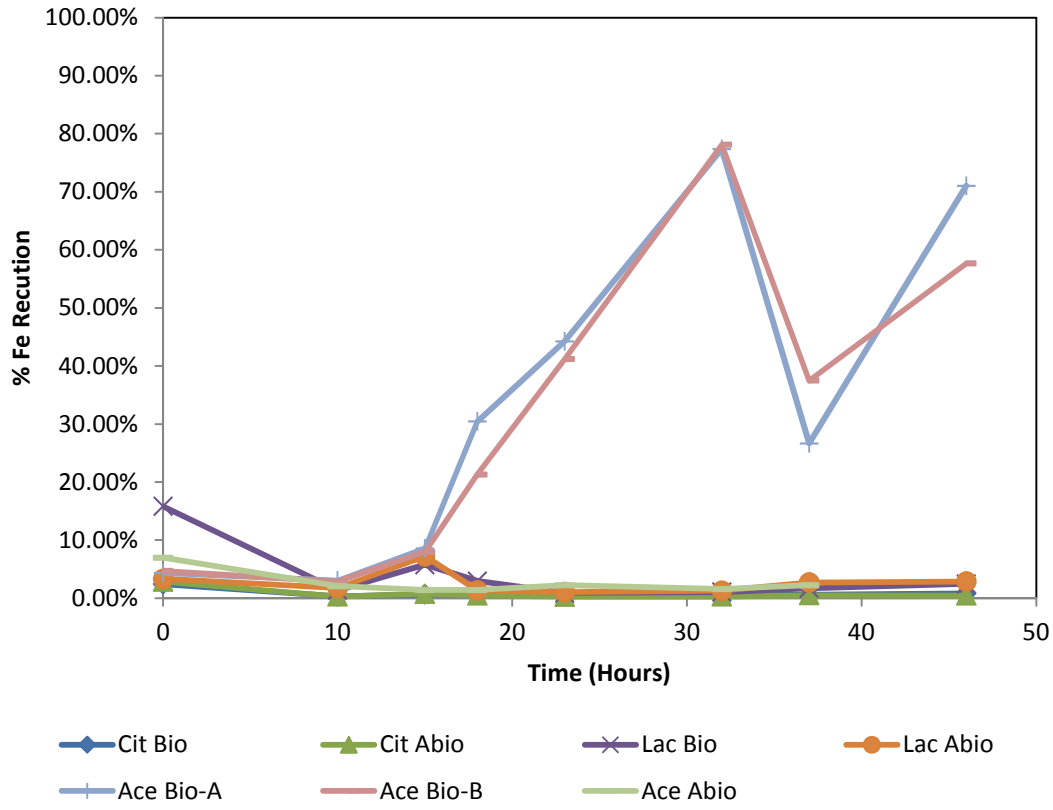

Figure 3: Percent iron reduction for abiotic (Abio) and biotic (Bio) FeCit (10 mM) samples with varying electron donor. For the acetate electron donor there were two biotic samples named A and B. Iron reduction was determined from a ratio of iron (II) over total iron.

Iron concentration and electron donor data for the citrate and lactate bottles (Figures A9 to A12) show similar results for biotic and abiotic samples, concluding that GS-15 was unable to couple oxidation of citrate or lactate with Fe (III) reduction to maintain growth.

3.3 Sampling of Solid Goethite (E33) with Three Electron Donors

This sampling process was done to determine the electron donors metabolism and growth rates for GS-15 using E33 (50 g/L). The data from these samples was to be compared to the growth and iron reduction seen for Fe-cit (10 mM) and As-E33 (50 g/L). These samples were inoculated with Son-3A, (Figure A5). The only electron donor

metabolized by GS-15, and the only iron reduction, occurred in E33 (50 g/L) with citrate (Figure 4 and Figure 5). This data did not coordinate with literature, as GS-15 has not shown the ability to metabolize citrate as an electron donor (Lovely, 1993); contamination was suspected so further comparisons were not made.

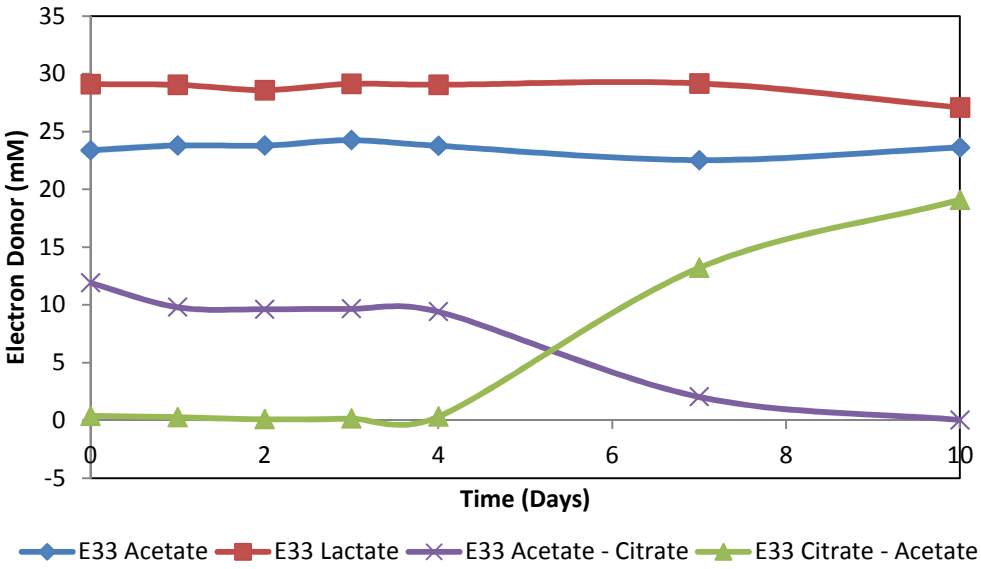

Figure 4: Electron donor concentration for GS-15 biotic E33 (50 g/L) sampling for lactate (10 mM), acetate (10 mM), and citrate (10 mM). For the citrate bottle, both the acetate and citrate concentrations have been listed.

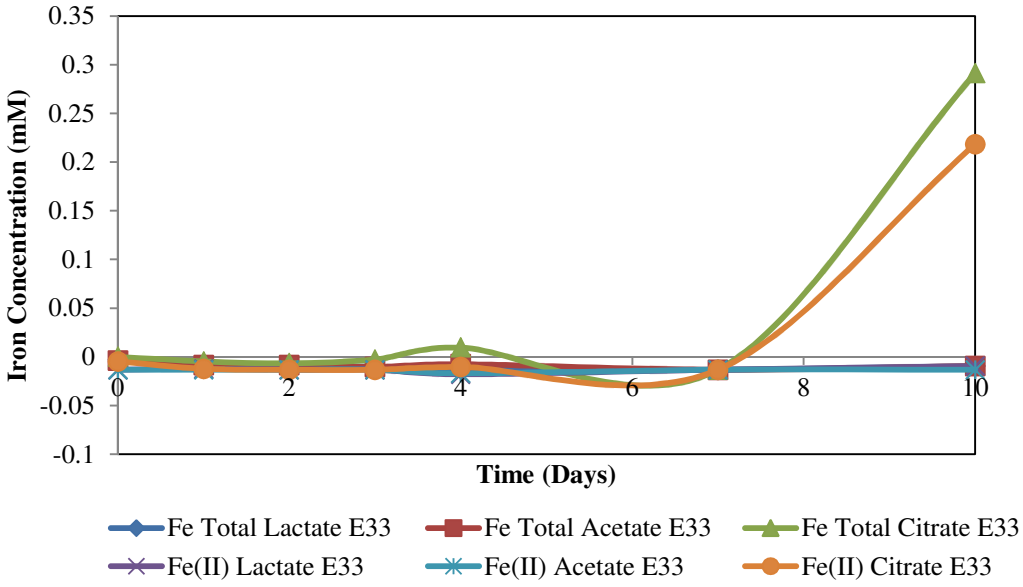

Figure 5: Iron concentration for GS-15 biotic E33 (50 g/L) sampling for lactate (10 mM), acetate (10 mM), and citrate (10 mM), which were determined using the Ferrozine method.

3.4 Sampling of Dissolved Sodium Arsenate with Acetate (10 mM)

This sampling was done to confirm whether GS-15 was incapable of reducing arsenic as shown by Tadanier (2005). During testing to see if GS-15 could reduce sodium-arsenate, no acetate was metabolized (Figure 6). These samples were inoculated with Son-3A, (Figure A5). These cells were also used to inoculate the E33 (50 g/L) samples, of which contamination was suspected; therefore, these results are not conclusive even though they show results that coordinate with literature (Tadanier, 2005).

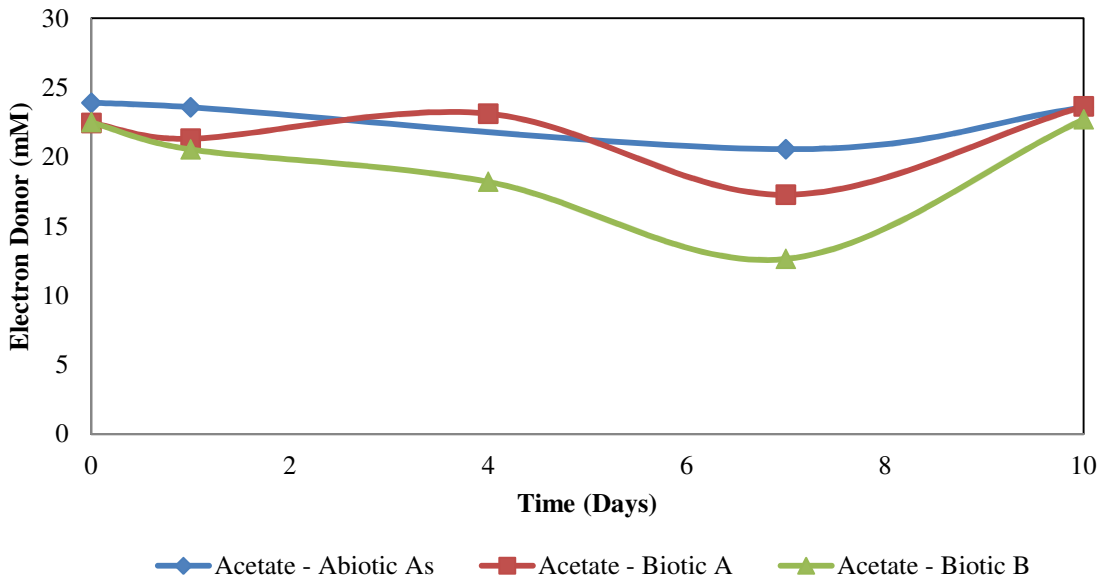

Figure 6: Electron donor metabolism for GS-15 in sodium-arsenate (10 mM) and acetate (10 mM) media.

3.5 Arsenic Loaded Solid Goethite with Acetate (10 mM)

Through the sampling of the As-E33 (50 g/L) with acetate (10 mM) as the electron donor it was seen that the percent iron reduction was 65% (Figure A13). 35% of the acetate was metabolized (Figures A14). These samples were inoculated with Son-4B and 5B at the same time (Figure A5). The arsenic release followed a trend of arsenic release to approximately day seven, followed by a period of decreased arsenic release suggesting adsorption occurred. The maximum release for this system was 1.20 µM on day five. This amount was reduced by 24% during the adsorption period (Figure 7), which was lower than the 40 CFR 261 standard. This maximum release is 0.024% of the total arsenic (5 mM) in the system. Arsenic release in the abiotic sample was 0.8 µM lower than when GS-15 was present (Figure A15).

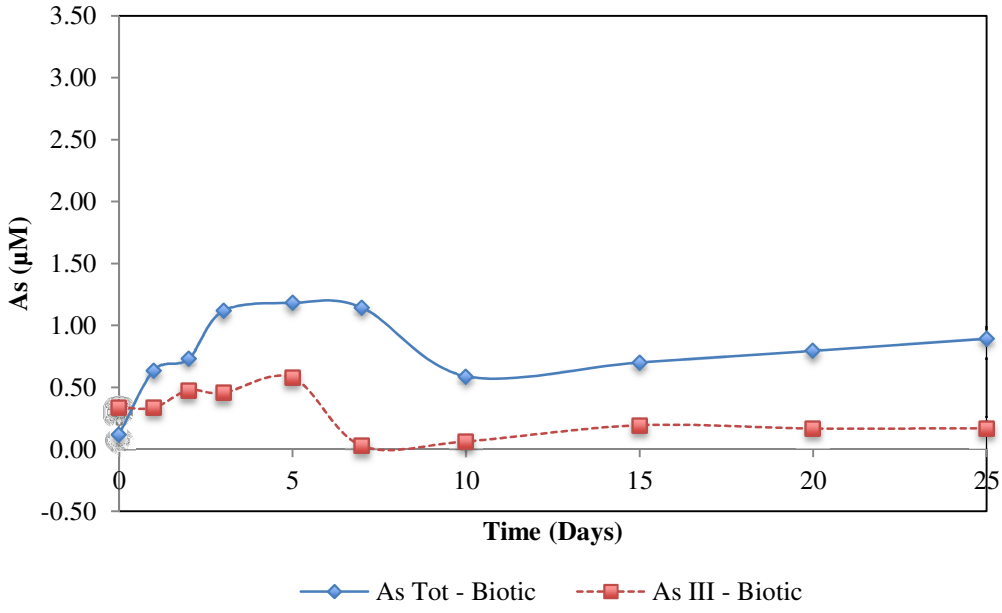

Figure 7: Arsenic concentration for the As-E33 (50 g/L) with acetate (10 mM) as the electron donor.

3.6 Arsenic Loaded Solid Goethite with Citrate (10 mM)

Through the sampling of the As-E33 (50 g/L) with citrate (10 mM) as the electron donor, it was seen that the citrate was metabolized completely to produce 13 mM of acetate (Figure A17). These samples were inoculated with Son-4B and 5B at the same time, (Figure A5). These were the same cells used to inoculate As-E33 (50 g/L) with acetate (10 mM).

The metabolism of citrate by GS-15 has not been shown to occur in literature (Lovley, 1993), therefore contamination was suspected. The iron was reduced by 90% (Figure A16). The arsenic release was characterized by a rapid initial arsenic release of 2.9 µM on day three (Figure 8), followed by a reduction in arsenic to 0.5 µM by day seven. This reduction in arsenic is also in coordination with a darkening in the solid

media. The maximum was lower than the 40 CFR 261 standards. This maximum release is 0.058% of the total arsenic (5 mM) in the system. The release of arsenic in the abiotic bottle achieved a maximum of 3.12 µM, which was more than that released with the presence of GS-15 (Figure A18). Comparison of this system to that of acetate (10 mM) as the electron donor; the maximum arsenic release of citrate (10 mM) is 1.92 µM greater. In addition, adsorption of arsenic in systems with citrate occurred at a higher percentage, 84%, versus 24% for acetate.

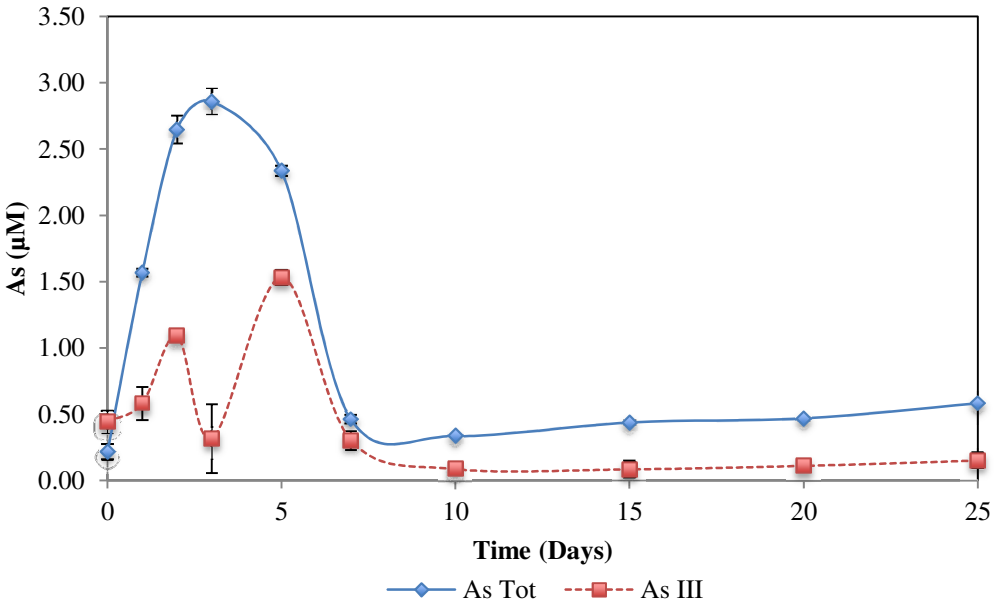

Figure 8: Arsenic concentration for the As-E33 (50 g/L) with citrate (10 mM) as the electron donor.

3.7 Arsenic Loaded Solid Goethite with Acetate (10 mM) and Elevated Phosphate (3.2 mM)

Through the sampling of the As-E33 (50 g/L) with acetate (10 mM) as the electron donor and elevated phosphate (3.2 mM) to increase growth, it was seen that the

iron was reduced by 75%, (Figure A19) and acetate metabolism was 80% (Figure A20). The arsenic release reached a maximum of 31.0 µM at day three (Figure 9), followed by a reduction in arsenic to 2.5 µM. This reduction in arsenic is also in coordination with a darkening in the solid media with suspended particles that did not settle with time.

The arsenic release in the abiotic system reached a maximum of 23.8 µM (Figure A21), 7.2 µM less than the biotic system. This maximum release is 0.62% of the total arsenic in the system. Comparison of this system to that with acetate (10 mM) as the only amendment shows an increase in iron reduction by 10%, an increase in acetate metabolism by 45%, and an increase of arsenic release by 96%. These can be asserted to the increased cell growth as phosphate was metabolized during cell synthesis.

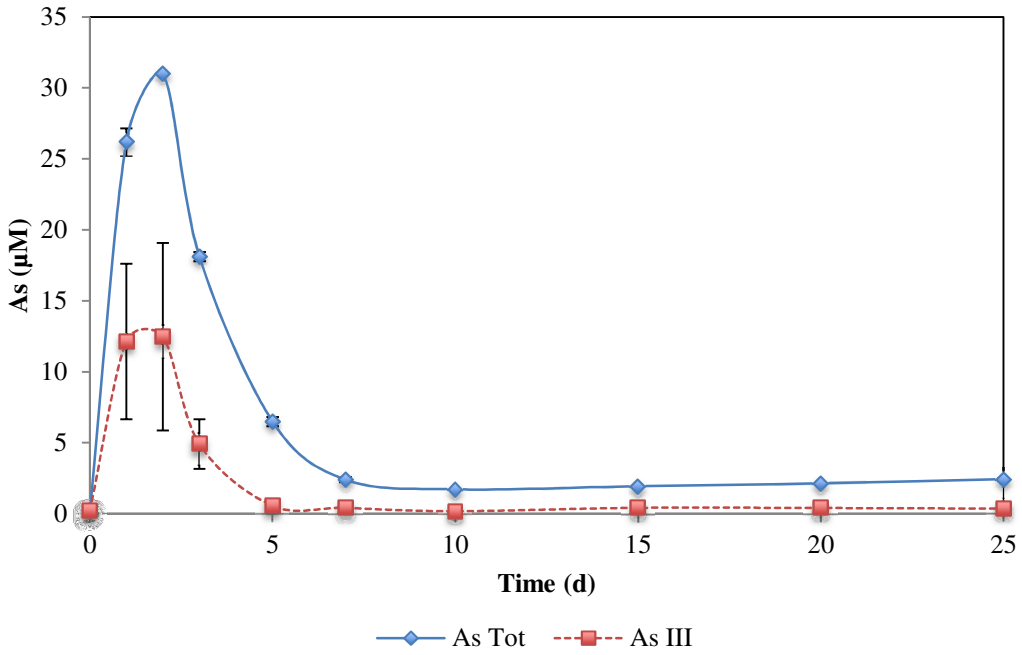

Figure 9: Arsenic concentration for the arsenic loaded solid goethite with acetate (10 mM) as the electron donor and elevated phosphate (3.2 mM).

CHAPTER 4: CONCLUSION

Arsenic as a human carcinogen is of major concern when it occurs in elevated concentration in supply systems. IBS media has been identified as one of the most promising treatment methods as it has the fewest water quality requirements for pre-treatment. This media is considered a one-time use media and has been identified under the 40 CFR 261 EPA regulation to be a non-hazardous waste that can be disposed of in municipal landfills. However, studies have shown that arsenic has been released to landfill leachate. Therefore, more research is necessary to understand what causes this release.

Release of arsenic in landfill leachate due to spent IBS is not greatly understood because of the chemical and biological processes that it is exposed to once entering a landfill. Through this research, insight into arsenic release due to iron reducing microbes was gained. This research showed that GS-15 did not cause arsenic to be released at a level that would violate regulation 40 CFR 26. It was seen that for all amended solutions tested that the initial release of arsenic happened within seven days, followed by a reduction in arsenic in solution. This reduction of arsenic in solution was coordinated with a change in color and properties of the solid media. Further research will need to be conducted to determine the final iron mineral types in order to determine if the reduction of arsenic in the system is adsorption or sequestration.

It was seen that under the condition of synthetic leachate with citrate (10 mM) as the electron donor that the arsenic release was less than in abiotic conditions; however, arsenic release with acetate (10 mM) as the electron donor resulted in more release than in the abiotic systems. Ten times the release of arsenic was seen for the samples when elevated phosphate (3.2 mM) was with acetate (10 mM) as the electron donor.

Further research will need to be conducted as contamination was suspected for the two As-E33 samples with acetate and citrate as the electron donors, as GS-15 has not been shown to be capable of metabolizing citrate. However, as arsenic was not reduced these results still allow for some assumptions to be made about the effects of iron reducing microbes on the release of arsenic from spent IBS media. It can then be concluded that iron-reducing microbes allow for initial arsenic release for at the most seven days, peaking at day 3, before sequestration processes reduced the arsenic to levels below 2.5 μM (0.19 mg/L). Arsenic release was never above 0.65% of the total arsenic in the system. Therefore, iron-reducing microbes do not cause significant release of arsenic from spent IBS media in landfill conditions that would result in toxicity characteristics to be violated. Further research should be done to determine the effects that the other identified competitive anions have as these anions could lead to further release of arsenic depending on the affinity of the anion for the iron media.

APPENDIX: Figures and Tables

Table A1: Table of arsenic treatment technologies water quality requirements (US EPA, 2003).

Factors	Sorption Processes		
	Ion Exchange	**Activated Alumina**	**Iron Based Sorbent Media**
Removal Efficiency	95%	95%	Up to 98%
Total Water Loss	1-2%	1-2%	1-2%
Pre-Oxidation Required for As(III)	Yes	Yes	Yes
Optimal Water Quality Condensations	pH 6.5-9 < 5 mg/L NO_3^{-1} < 50 mg/L SO_4^{-2} < 500 mg/L TDS < 0.3 NTU Turbidity	pH 5.5-6 pH 6-8.3* < 250 mg/L Cl^{-1} < 2 mg/L F^{-1} < 360 mg/L SO_4^{-2} < 30 mg/L Silica < 0.5 mg/L Fe^{+3} < 0.05 mg/L Mn^{+2} < 1,000 mg/L TDS < 4 mg/L TOC < 0.3 NTU Turbidity	pH 6-8.5 < 1 mg/L PO_4^{-3} < 0.3 NTU Turbidity

Table A2: Composition of synthetic leachate for GS-15.

Component	Concentration (g/L)
$NaHCO_3$	2.52
NH_4Cl	0.59
$NaH_2PO_4 \cdot H_2O$	0.0029
KCl	0.38
$CaCl_2$	0.22
$MgCl_2 \cdot 6\,H_2O$	0.85
Na_2SiO_3 (27% SiO_2)	27.5 mL/L
Na_2SO_4	0.11
Wolfe's Minerals	10 mL/L
Wolfe's Vitamins	10 mL/L

Table A3: Composition of ATCC® #1768 growth media for GS-15.

Component	Concentration (g/L)
Ferric citrate (Sigma F-6129)	13.70
$NaHCO_3$	2.50
NH_4Cl	0.25
$NaH_2PO_4 \cdot H_2O$	0.60
KCl	0.1
Sodium Acetate	6.8 g
Wolfe's Vitamins	10 ml/L
Wolfe's Minerals	10 ml/L

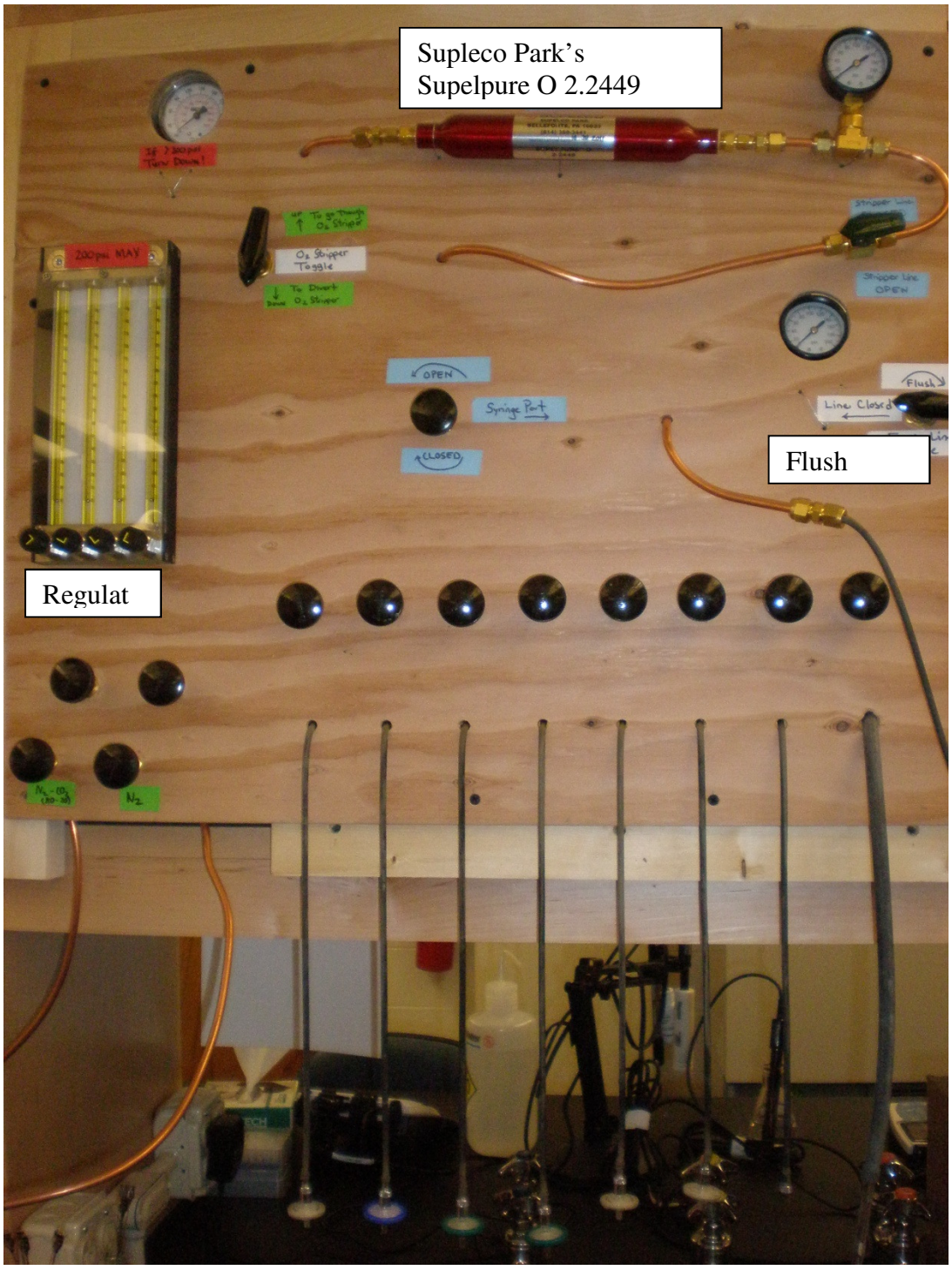

Figure A1: Picture of gassing station used to deoxygenate media and do anaerobic sampling.

Table A4: Media composition used for synthesis of As-E33.

Component	Concentration (g/L)	Anion	Concentration (mM)
E33 Solids	100		
Na_3AsO_4	3.13	As	10.04
Na_2HPO_4	0.55	P	3.84
Na_2SO_4	0.07	S	0.47
Na_2SiO_3 27% SiO_2	1.55	Si	9.68
$NaHCO_3$	0.25	Bicarbonate	3.00

Figure A2: Picture of color change of synthetic leachate with dissolved Iron (III) citrate. Left fresh media with approximately 5% iron reduction right is twenty-four hour old media with approximately 80% iron reduction.

Figure A3: Picture of original color of synthetic leachate with FeCit.

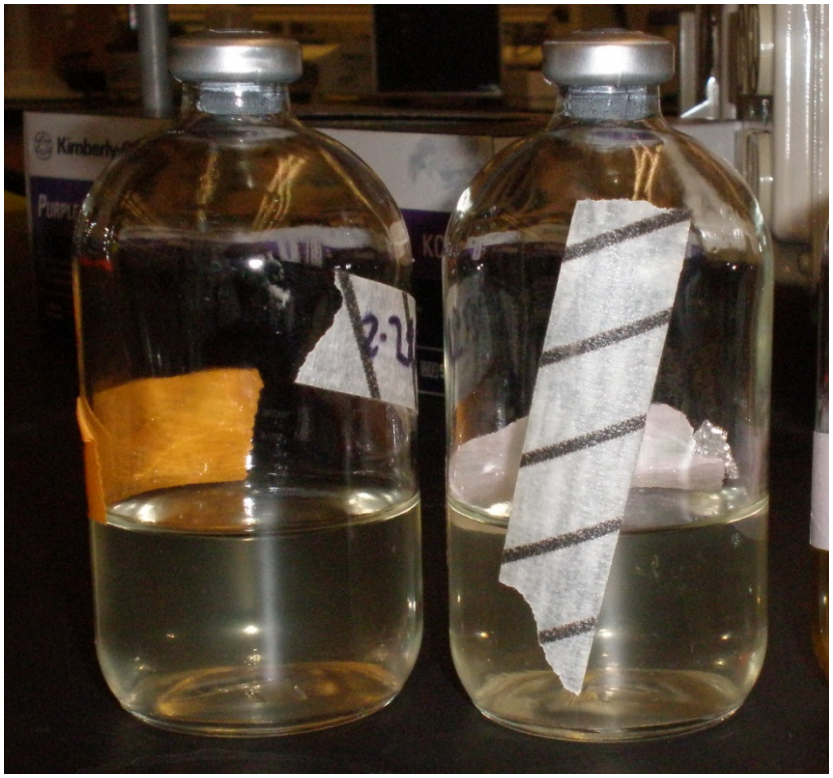

Figure A4: Picture of color change of synthetic leachate with FeCit when reduction is approximately 80%.

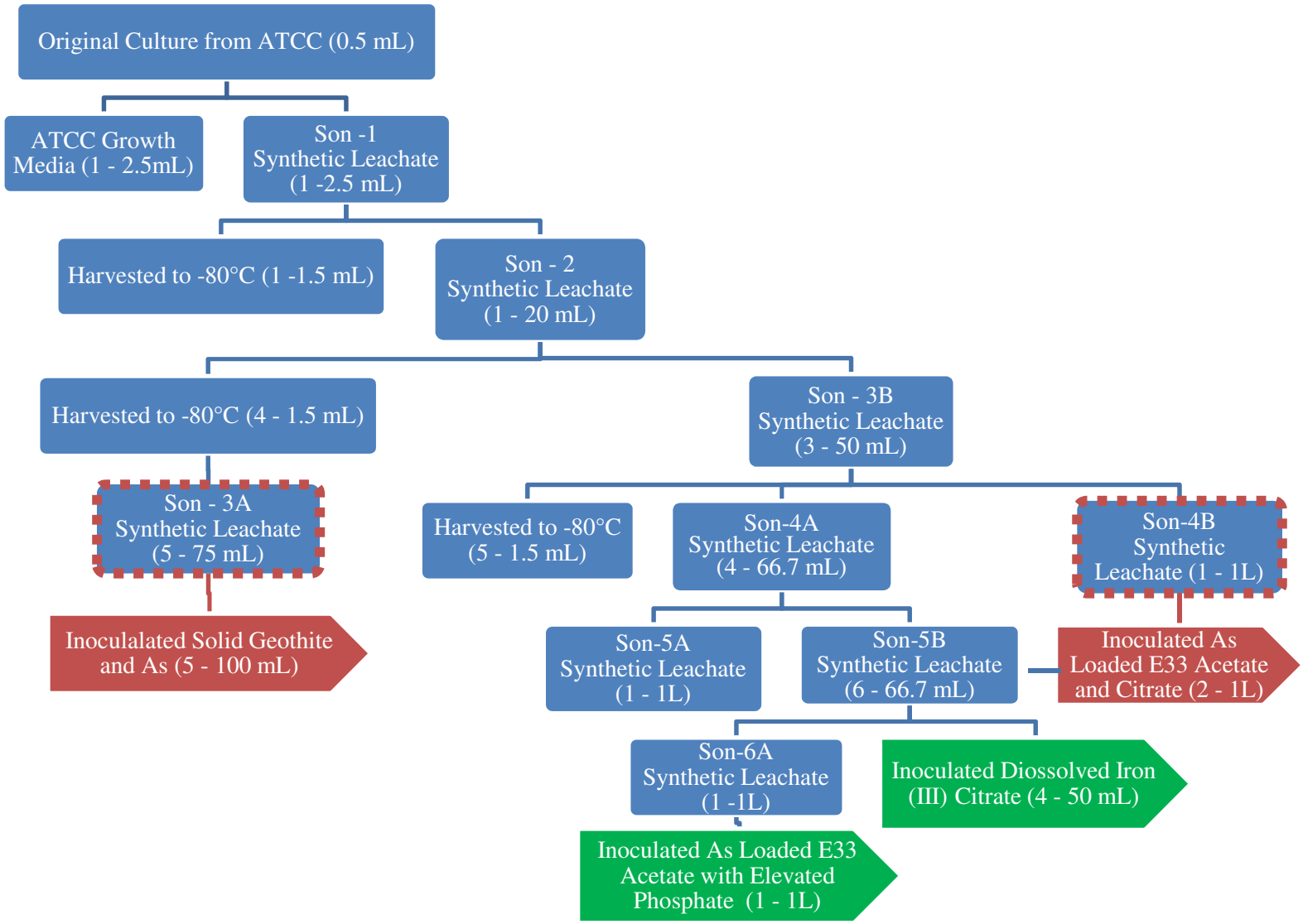

Figure A5: A summary of all transfers done from the original culture to inoculation of all samples. The arrow shaped boxes show boxes that were used to inoculate samples for testing. The green arrows represent samples that did not appear to have contamination, while the red appear to have contamination. The red outlined boxes show the likely point of contamination.

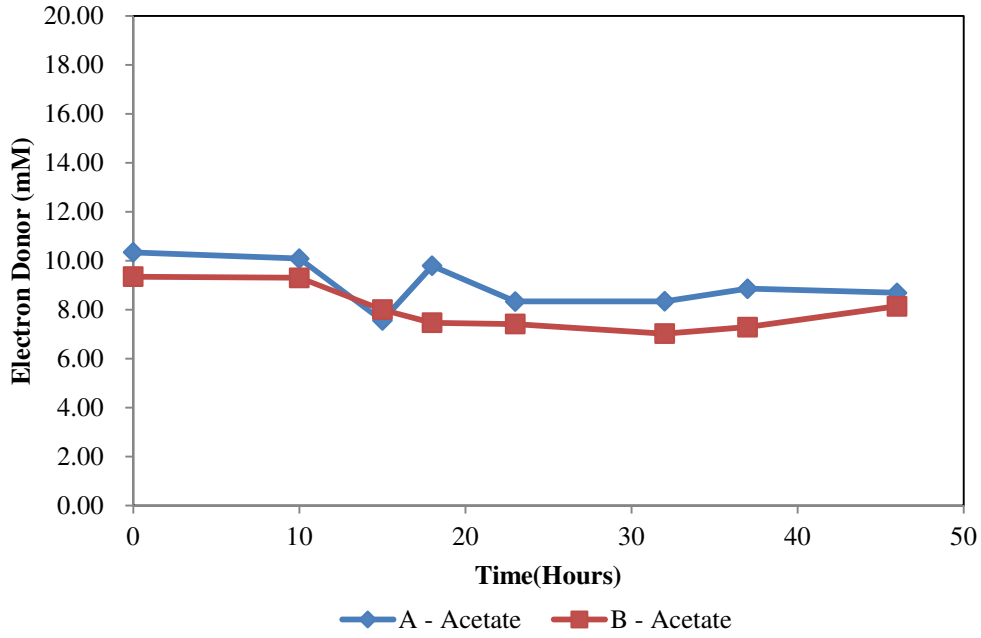

Figure A6: Electron donor concentration for biotic FeCit bottles with acetate (10 mM) as the electron donor. Note there were two biotic bottles that were named A and B.

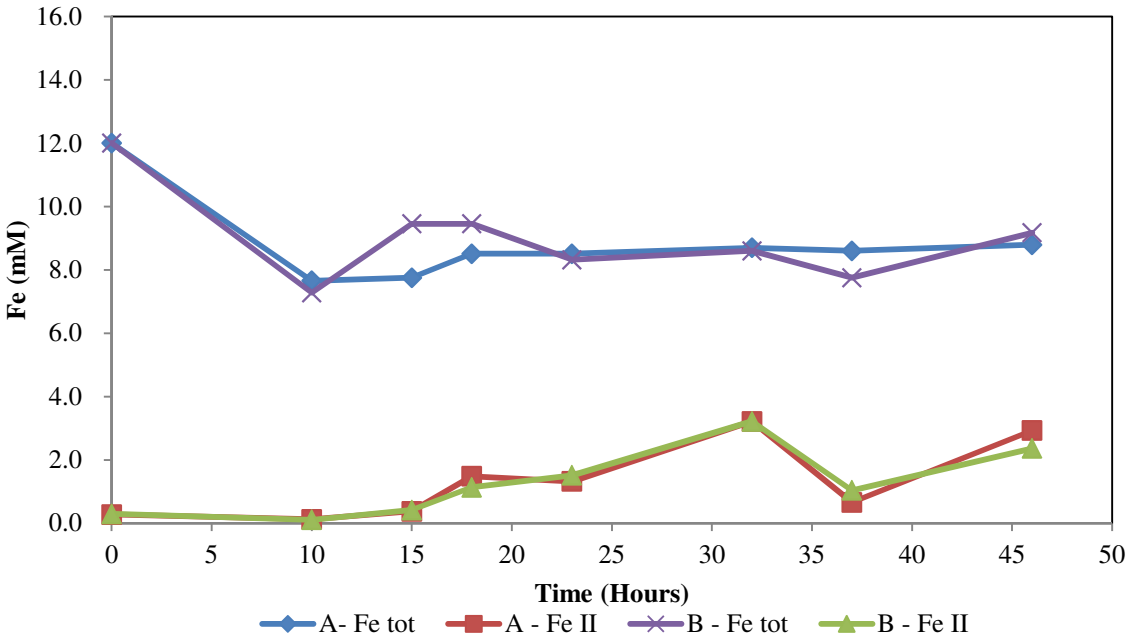

Figure A7: Iron concentrations for the biotic FeCit bottles with acetate (10 mM) as the electron donor. Note there were two biotic bottles that were named A and B.

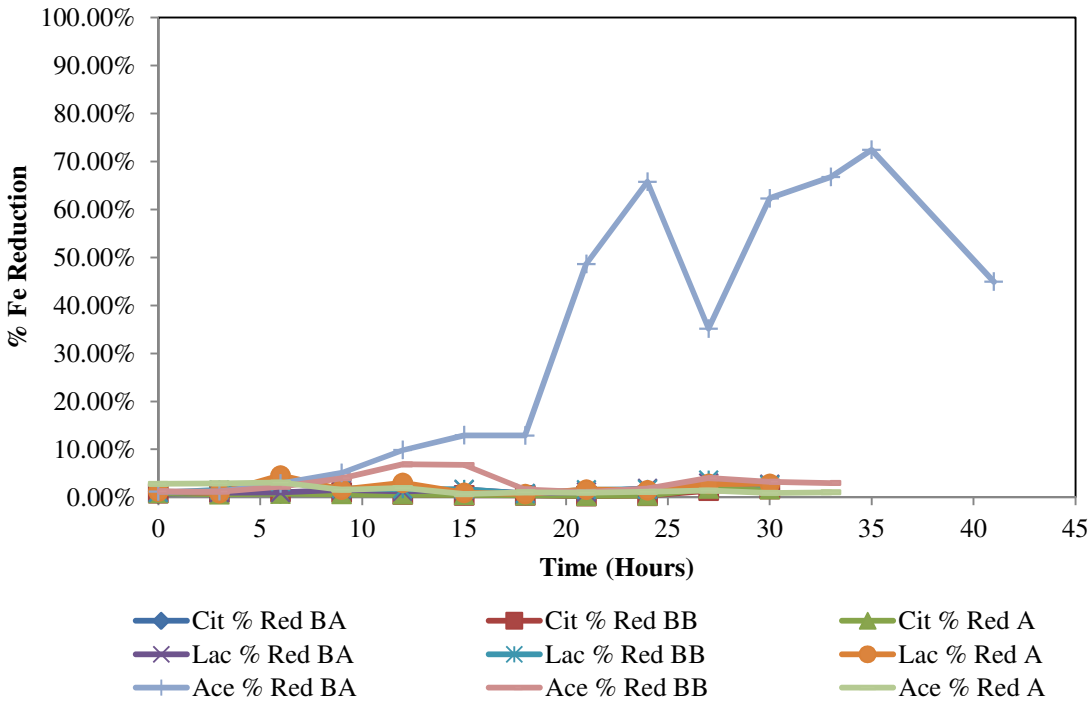

Figure A8: Percent in iron reduction for preliminary growth rates showing the repeated decrease in iron reduction, which occurred here at 27 hours and 41. Note there were two biotic bottles for each electron donor that were named BA and BB and one abiotic bottle that was named A

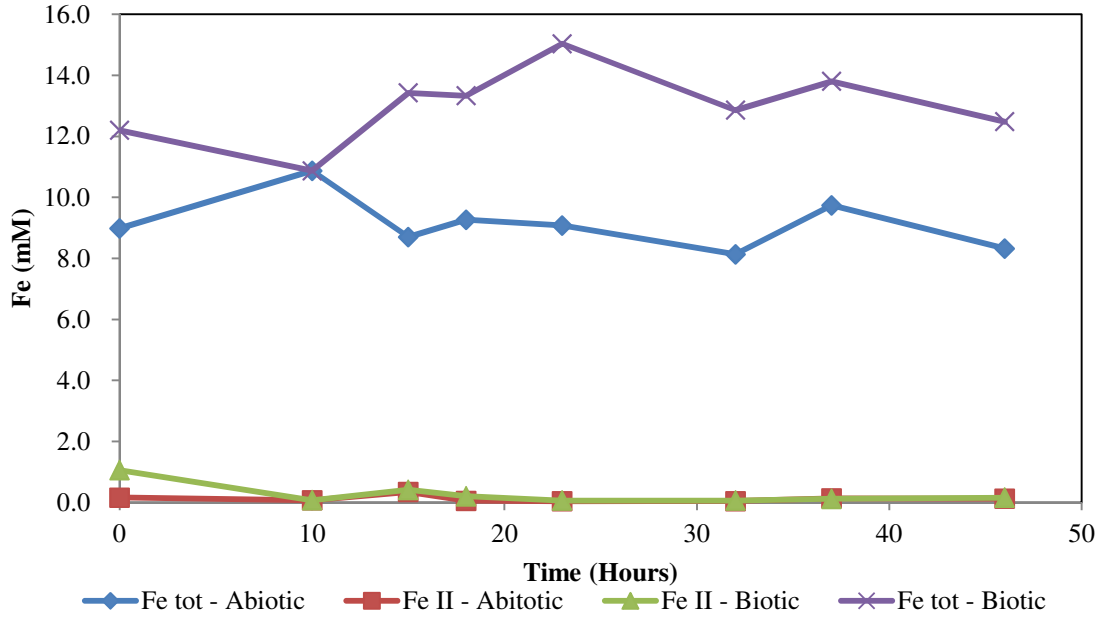

Figure A9: Iron concentration for the biotic and abiotic FeCit samples with lactate (10 mM) as the electron donor, where no iron reduction occurred.

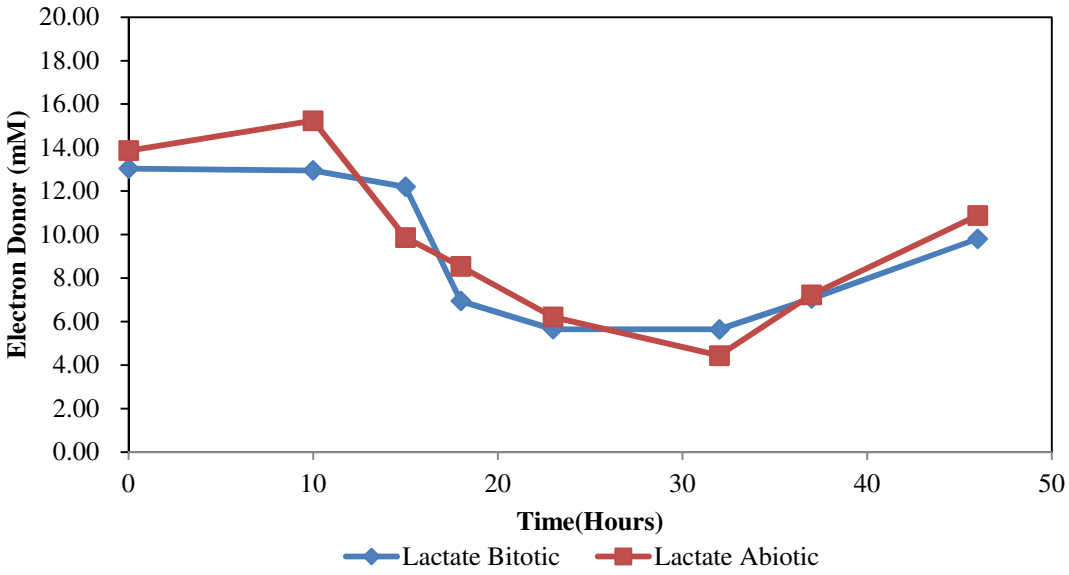

Figure A10: Electron donor concentration for biotic and abiotic FeCit bottles with lactate (10 mM) as the electron donor, where the biotic and abiotic electron donor concentration is similar showing no metabolism by GS-15.

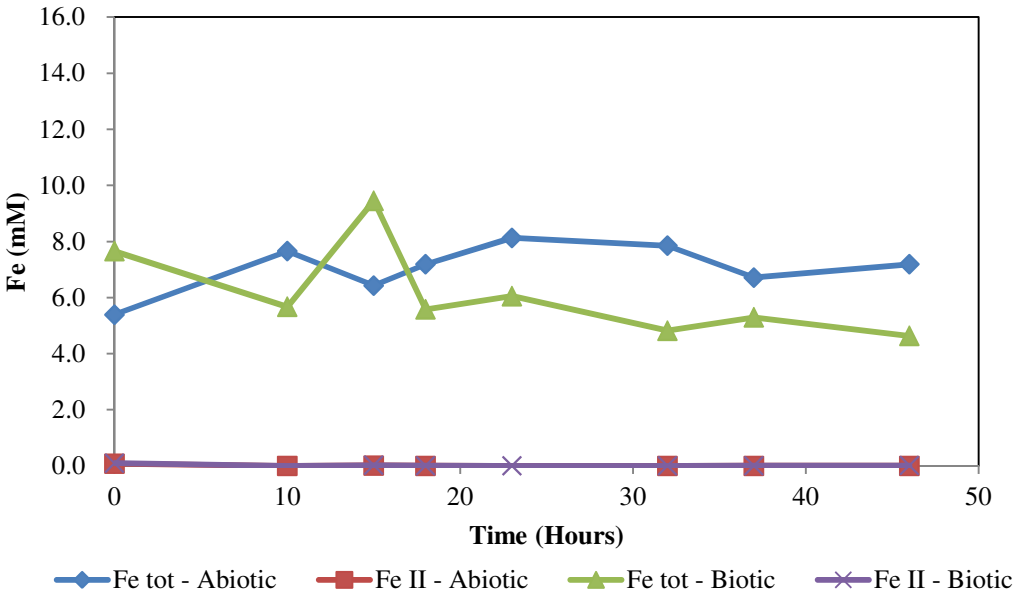

Figure A11: Iron concentration for the biotic and abiotic FeCit samples with citrate (10 mM) as the electron donor, where no iron reduction occurred.

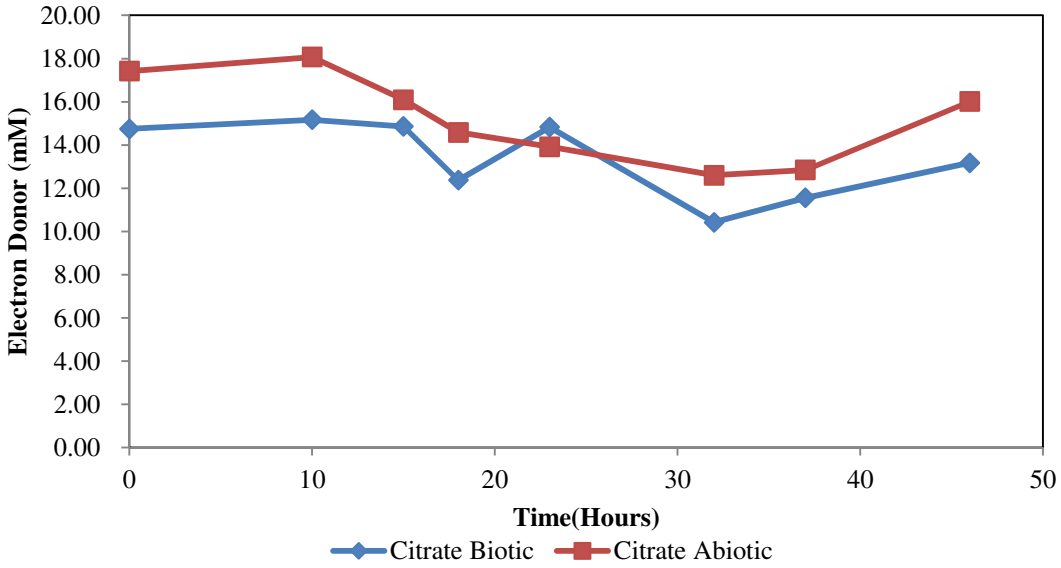

Figure A12: Electron donor concentration for the biotic and abiotic FeCit bottle with citrate (10 mM) as the electron donor, where the biotic and abiotic electron donor concentrations were similar showing no metabolism by GS-15.

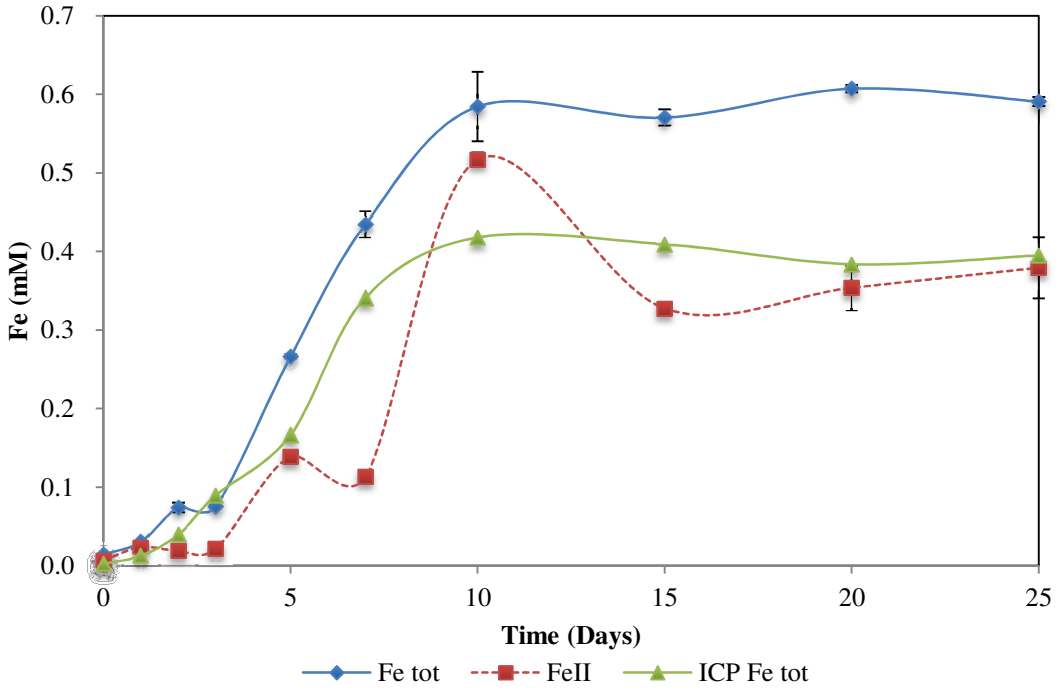

Figure A13: Iron concentrations in the As-E33 bottle with acetate (10 mM) as the electron donor, where Fe tot and Fe II were determined from the Ferrozine method (digestion by HCl) and ICP Fe tot is determined using a Varian Vista AX ICP-AES (digestion by 5% nitric acid).

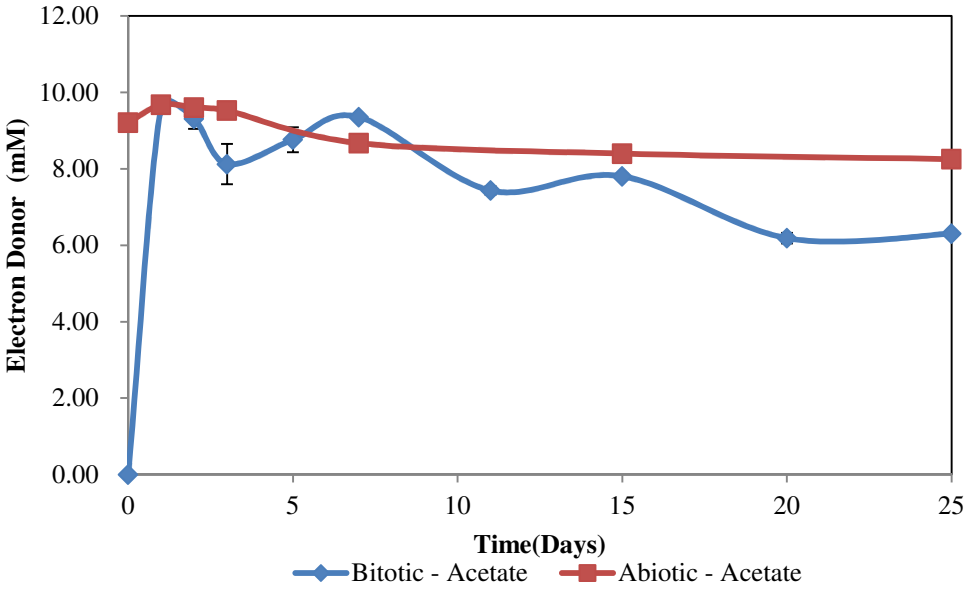

Figure A14: Electron donor concentration for the biotic and abiotic As-E33 bottle with acetate (10 mM) as the electron donor, where acetate is metabolized by GS-15. Acetate was present in the biotic bottle at day zero.

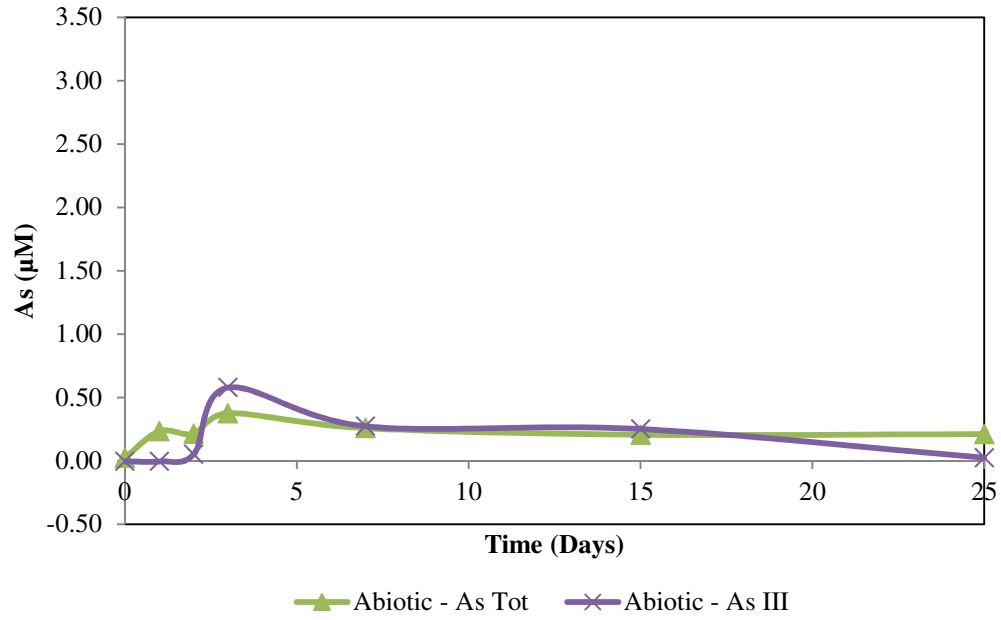

Figure A15: Arsenic concentration for the abiotic As-E33 bottle with acetate (10 mM) as the electron donor, showing that arsenic release was lower without the presence of GS-15.

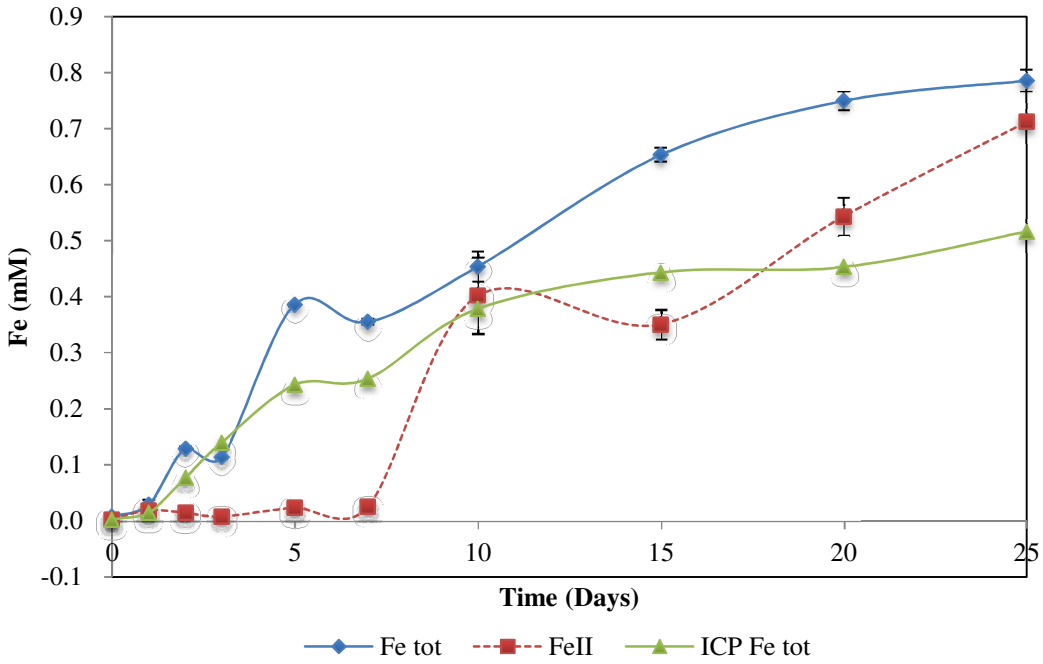

Figure A16: Iron concentrations in the arsenic loaded solid goethite bottle with citrate (10 mM) as the electron donor, where Fe tot and Fe II were determined from the Ferrozine method (digestion by HCl) and ICP Fe tot is determined using a Varian Vista AX ICP-AES (digestion by 5% nitric acid).

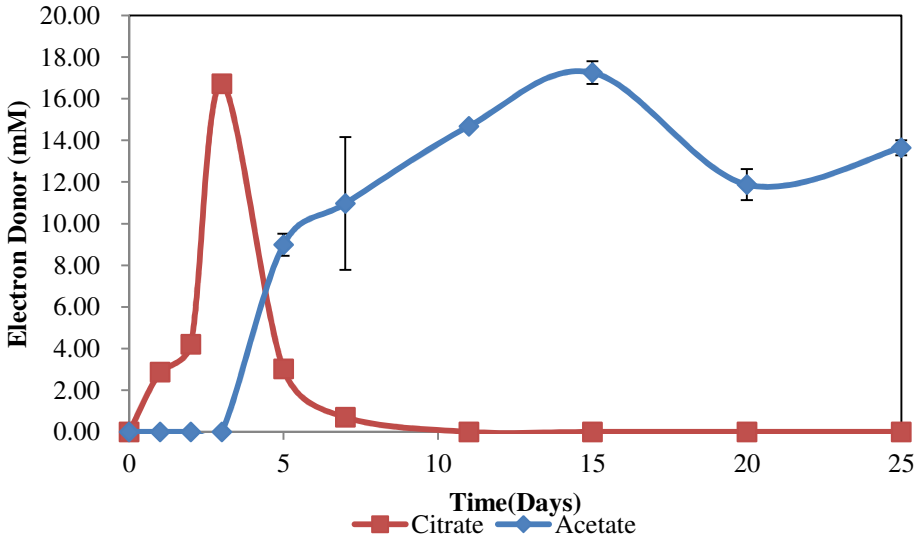

Figure A17: Electron donor concentration for the biotic As-E33 bottle with citrate (10 mM) as the electron donor, where citrate is metabolized by GS-15 to acetate. There was citrate present in the system on day zero.

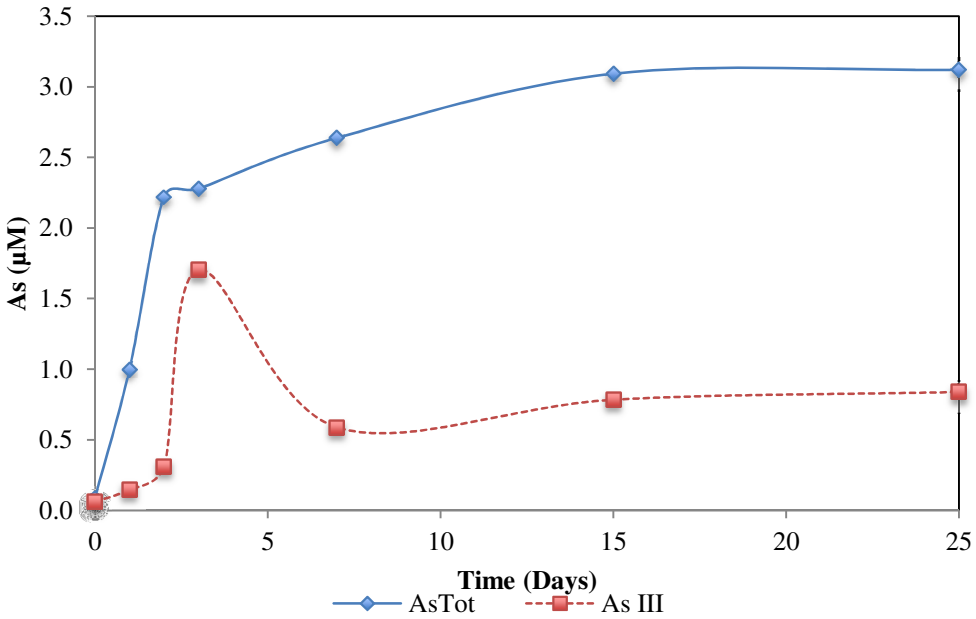

Figure A18: Arsenic concentration for the abiotic As-E33 bottle with citrate (10 mM) as the electron donor, showing that arsenic release was higher without the presence of GS-15.

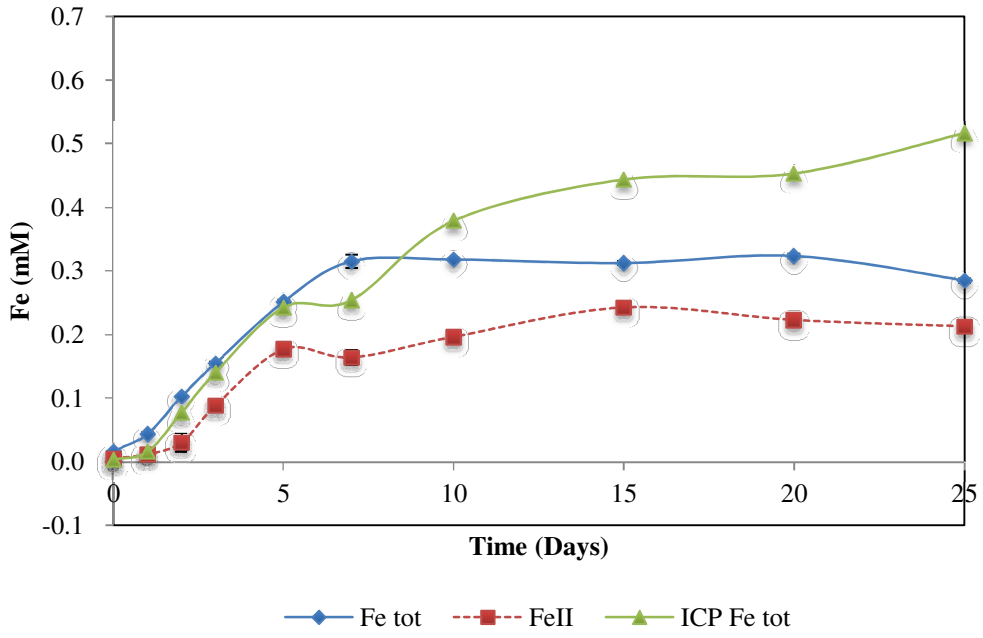

Figure A19: Iron concentrations in the biotic As-E33 bottle with acetate (10 mM) as the electron donor and elevated phosphate (3.2 mM), where Fe tot and FeII were determined from the Ferrozine method (digestion by HCl) and ICP Fe tot is determined using a Varian Vista AX ICP-AES (digestion by 5% nitric acid).

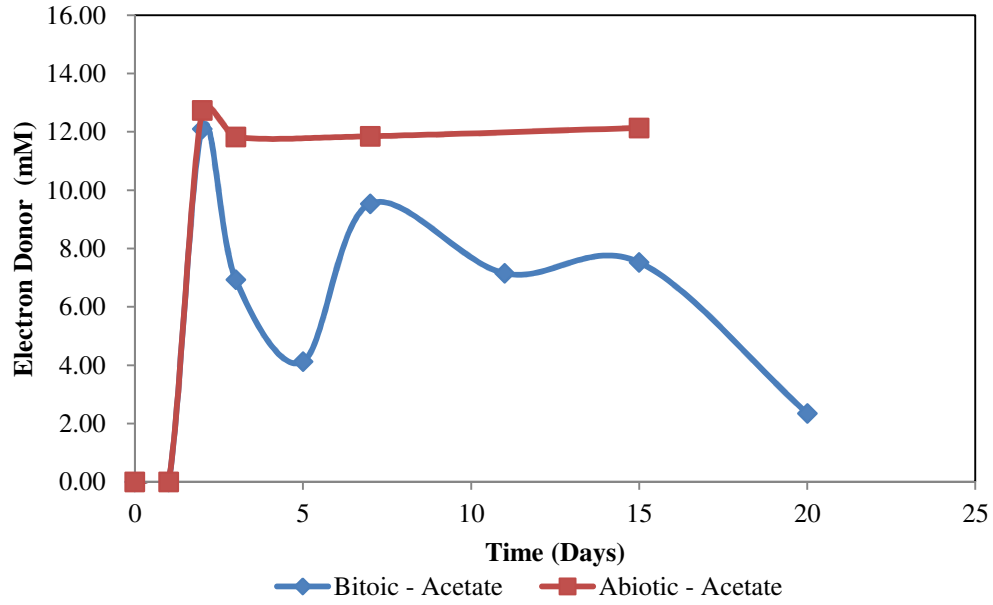

Figure A20: Electron donor concentration for the biotic and abiotic As-E33 bottle with acetate (10 mM) as the electron donor and elevated phosphate (3.2 mM), there was acetate present in the system on day zero. Samples for day twenty-five were not measurable due to equipment error and are not shown.

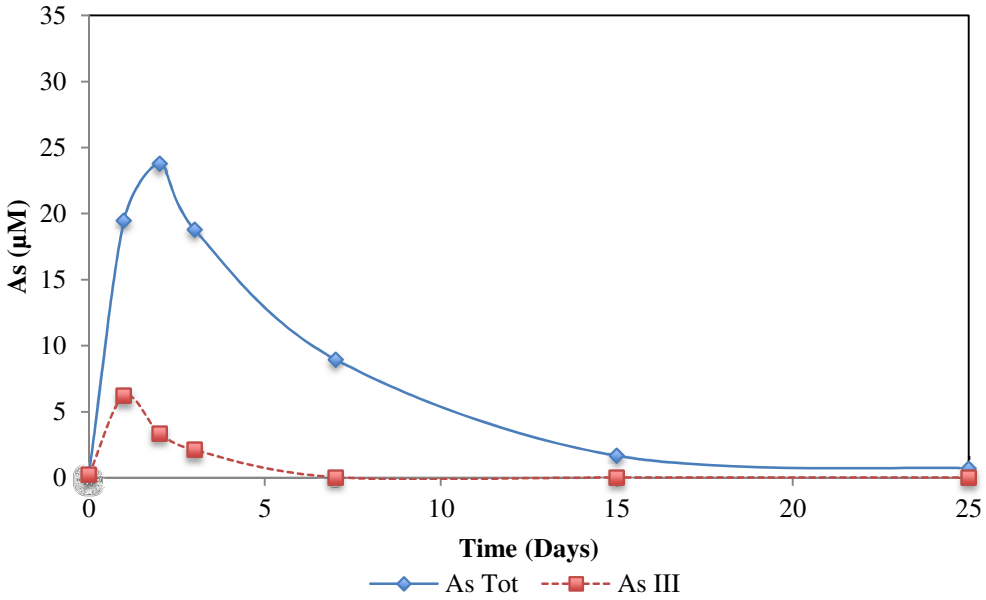

Figure A21: Arsenic concentration for the abiotic As-E33 bottle with acetate (10 mM) as the electron donor and elevated phosphate (3.2 mM), showing that arsenic release is lower without the presence of GS-15.

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